Bm

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 982 037 A1

(12) EUROPEAN PATENT APPLICATION
published in accordance with Art. 158(3) EPC

(43) Date of publication:
01.03.2000 Bulletin 2000/09

(21) Application number: 99909244.8

(22) Date of filing: 19.03.1999

(51) Int. Cl.^{7}: A61K 38/17

(86) International application number:
PCT/JP99/01373

(87) International publication number:
WO 99/47155 (23.09.1999 Gazette 1999/38)

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB IT LI NL SE

(30) Priority: 19.03.1998 JP 7091498

(71) Applicant:
SNOW BRAND MILK PRODUCTS, CO., LTD.
Sapporo-shi Hokkaido 065-0043 (JP)

(72) Inventors:
- TANI, Tohru
Kusatsu-shi Shiga-ken 525-0055 (JP)
- KONDO, Hiroyuki
Otsu-shi Shiga-ken 520-2143 (JP)

(74) Representative:
Wakerley, Helen Rachael
Reddie & Grose,
16 Theobalds Road
London WC1X 8PL (GB)

(54) PREVENTIVES AND/OR REMEDIES FOR SEPSIS

(57) Novel preventives and/or remedies for sepsis. The above preventives and/or remedies contain as the active ingredient tumor cytotoxic factor-II (TCF-II) and exert particularly excellent effects on sepsis caused by at least one factor selected from the group consisting of infectious disease, burn, operation, cancer, acquired immunological deficiency syndrome (AIDS), radiation therapy, chemotherapy and total parenteral nutrition (TPN).

EP 0 982 037 A1

Description

Technical field

[0001] The present invention relates to a therapeutic agent for preventing and/or treating sepsis, comprising Tumor Cytotoxic Factor-II (TCF-II) as an effective ingredient. By the present invention, a therapeutic agent for preventing and/or treating sepsis caused by more than one factor selected from the group consisting of infectious disease, burn, surgery, cancer, acquired immunodeficiency syndrome (AIDS), radiotherapy, chemotherapy, long term total parenteral nutrition (TPN) is provided and useful as a medicine.

Prior art

[0002] Sepsis is generally severe systemic infectious disease which developes by continuous or intermittent invasion of bacteria or products secreted by them into blood from bacterial infectious focus present somewhere in tissue or organ of human body. Sepsis is induced by chemotherapy using corticosteroid or antitumor agent, etc., radiotherapy like cobalt radiation, or treatment or operation such as self-retaining catheter, blood dialysis, organ transplantation, heart surgery etc. in a patient with malignant tumor, leukemia, malignant lymphoma, AIDS, collagen disease, renal insufficiency, hepatic disease, cerebrovascular disease or diabetes or in a host with decreased resistance such as humoral immunodeficiency or cellular immunodeficiency of an aged person or an immature infant. Recently, though various kinds of antibiotic have been developed and clinically used for the treatment of sepsis, various kinds of antibiotic-resistant bacterium, such as penicillin- or methicillin-resistant Staphylococcus aureus, emerged, so that mortality rate of patients with sepsis is still about 25 % and many patients die due to sepsis every year.

[0003] As bacteria which induce sepsis, in addition to the afore- mentioned antibiotic-resistant bacteria, enterostreptococcus, gram-negative bacillus such as Escherichia coli, Pseudomonas aeruginosa, Klebsiella and Proteus, etc. are exemplified. Among these, a patient with sepsis due to gram-negative bacillus suffers from hyperthermia, chill trepidation, tachycardia, slight depression of blood pressure, depression of peripheral resistance, hyperkinemia, hyperventilation, respiratory alkalosis, hyperlactacidemia and sometimes heart failure at early stage, followed by depression of blood pressure and cardiac output and falling into severe conditions accompanying with metabolic acidosis, consiousness affection, acroagnosis, hypoglycemia and sometimes resulting in death. Therefore, treatment of sepsis is considered to be an important object.

[0004] Bacteria are mainly thought to invade into living body by two different kinds of route. One is exogenous case such as intravenous self-retaining catheter or puncture needle used on infusion or supplemental infusion, and the other is endogenous case such as invasion of bacteria widely distributed in human living body such as intestine etc.

[0005] Transfer of bacteria or products secreted by them from intestine is called as bacterial translocation or endotoxin translocation and is noticed as a trigger of inducing sepsis. Such a translocation of bacteria and/or products secreted by them from intestine is observed when a patient is severely damaged by burn, major surgery, etc, and when a patient after surgery or a patient with consciousness affection receives long term TPN treatment and becomes serious problems.

[0006] For the treatment of sepsis comprising these bacterial translocation, antibiotic treatment is chosen first and recognized to have a certain level of effects. However, antibiotic treatment is thought to have its limit due to its side-effects, appearance of antibiotic-resistant bacteria and its little effects on shock symptom. In addition, supplement of nutrition by infusion or treatment by γ-globulin is carried out but these are aimed for recovery of host immunity and remain as auxiliary treatment for antibiotic treatment. Thus, there is no effective treatment for sepsis.

Disclosure of the invention

[0007] The present inventors have eagerly studied to find out a therapeutic agent for sepsis and found that TCF-II known as tumor cytotoxic factor had an excellent effect for preventing and treating sepsis. Accordingly, an object of the present invention is to provide a therapeutic agent for preventing and/or treating sepsis caused by more than one factor selected from the group consisting of burn, surgery, cancer, AIDS, radiotherapy, chemotherapy and long term TPN, comprising TCF-II as an effective ingredient.

[0008] The present invention provides a therapeutic agent for preventing and/or treating sepsis, comprising TCF-II as an effective ingredient. The therapeutic agent of the present invention is useful for preventing and/or treating sepsis caused by more than one factor selected from the group consisting of burn, surgery, cancer, AIDS, radiotherapy, chemotherapy and long term TPN.

[0009] TCF-II is an effective ingredient of the present invention, a known protein derived from human fibroblast and has the following properties:

1) Molecular weight (SDS electrophoresis)

under non-reducing conditions;
78,000 ± 2,000 or 74, 000 ± 2, 000
under reducing conditions;
52,000 ± 2,000 (common band A)
30,000 ± 2,000 (band B)
26,000 ± 2,000 (band C)

2) Isoelectric point; 7.4-8.6

[0010] The above TCF-II can be obtained by a method of condensing culture medium of human fibroblast, adsorbing the condensate on ionexchange resin and purifying the eluent by affinity column chromatography (WO90/10651) or by a genetically engineered manipulation (WO92/01053).

[0011] TCF-II as an effective ingredient of the present invention can be TCF-II derived from human fibroblast IMR-90 or TCF-II genetically engineered using microorganism or other cell lines based on the genetic sequence described in patent publication WO90/10651. TCF-II obtained by genetically engineered manipulation described in patent publication WO92/01053 can be also used. In such a case, TCF-II with different polysaccharide chain or without polysaccharide chain due to the difference of host cell or microorganism can be used but TCF-II with polysaccharide chain is preferable. TCF-II obtained by these methods can be condensed and purified by usual isolation and purification method. For example, precipitation using organic solvent, salting-out, gel filtration, affinity chromatography using monoclonal antibody and electrophoresis can be exemplified. Purification by affinity chromatography using monoclonal antibody can use monoclonal antibody described in Japanese Published unexamined patent application No. 97 (1993). Obtained purified TCF-II can be lyophilized or kept freezed. Other substances showing the same activity as TCF-II can be used as the same therapeutic agent. For example, hepatocyte growth factor (HGF; Japanese Published unexamined patent application No. 22526/1988) which is different from TCF-II protein in five amino acids or Purified Scatter Factor (SF; Gherardi and Stocker, Nature, 346, 228 (1990)) can be exemplified.

[0012] The therapeutic agent of the present invention for preventing and/or treating sepsis can be administered intravenously, intramuscularly or subcutaneously as injections. These preparations can be produced according to a known pharmaceutical preparation method and pH conditioners, buffers, stabilizers can be added thereto if necessary. Dose of the therapeutic agent of the present invention for a patient depends on degree of symptom, health conditions, age and body weight of a objected patient. Though it is not especially restricted, 0.6-600 mg, preferably 6-60 mg, of purified TCF-II can be administered once a day for one adult patient.

Brief description of the drawings

[0013]

Figure 1 shows the effects of TCF-II on model animals of cecum-punctured sepsis in example 1.

- ● represents vehicle-administered group and
- ■ represents TCF-II-administered group

Figure 2 shows the effects of TCF-II on LPS-induced bacterial translocation in example 2.

- □ represents vehicle-administered group and
- ■ represents TCF-II-administered group

Best mode for carrying out the invention

[0014] The present invention will be described more specifically by the following examples but these are merely exemplified and the scope of the present invention is not restricted by these examples.

[Preparative example 1]

Purification of TCF-II

[0015] According to a method described in WO90/10651 and a method of Higashio et. al. (Higashio, K. et. al., B. B. R. C., vol. 170, pp397-404 (1990)), cell was cultured and purified TCF-II was obtained. That is, 3×10^6 human fibroblast IMR-90 (ATCC CCL186) cells were placed in a roller bottle containing 100 ml DMEM medium including 5 % calf fetal serum and cultured by rotating it at the rate of 0.5-2 rpm for 7 days. When the total number of cell reached 1×10^7 cells were deprived from the wall by trypsin digestion and collected at the bottom of bottle. And 100g of ceramic with the size of 5 - 9 mesh (Toshiba Ceramic) was sterilized and put therein, which was cultured for 24 hours. After then, 500 ml of the above culture medium was added thereto and the culture was continued. The total volume of culture medium was recovered every 7-10 days and fresh medium was supplemented. Production was kept for 2 months like this and 4 liters of culture medium was recovered per a roller bottle. Specific activity of TCF-II in culture medium obtained as above was 32 μ g/ml. Culture medium (750 L) was concentrated by ultrafiltration using membrane filter (MW 6,000 cut; Amicon) and purified by 4-steps chromatography, that is, CM- Sephadex C-50 (Pharmacia), Con-A Sepharose (Pharmacia), Mono S column (Pharmacia), Heparin-Sepharose (Pharmacia) to yield purified TCF-II.

[Preparative example 2]

Production of recombinant TCF-II

[0016] According to a method described in WO92/01053, cell transformed with TCF-II gene was cultured and purified TCF-II was obtained. That is, transformed Namalwa cell was cultured and 20 l of culture medium was obtained. This culture medium was treated by CM-Sephadex C-50 chromatography, Con-A Sepharose CL-6B chromatography and finally HPLC equipped with a Mono S column to yield about 11 mg of recombinant TCF-II.

[Preparative example 3]

Production of pharmaceutical preparation of TCF-II

[0017] An example of producing injections of TCF-II obtained preparative examples 1 and 2 was shown below/

(1)	TCF-II	20 μg
	human serum albumin	100 mg

[0018] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20 ml. Then, it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(2)	TCF-II	40 μ g
	Tween 80	1 mg
	Human serum albumin	100 mg

[0019] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(3)	TCF-II	20 μ g
	Tween 80	2 mg
	Sorbitol	4 g

[0020] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(4)	TCF-II	40 μ g
	Tween 80	1 mg
	Glycine	2 g

[0021] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(5)	TCF-II	40 μ g
	Tween 80	1 mg
	Sorbitol	2 g
	Glycine	1 g

[0022] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(6)	TCF-II	20 μ g
	Sorbitol	4 g
	Human serum albumin	50 mg

[0023] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(7)	TCF-II	40 μ g
	Glycine	2 g
	Human serum albumin	50 mg

[0024] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(8)	TCF-II	40 μ g
	Human serum albumin	50 mg

[0025] The above composition was dissolved in citric acid buffer solution with pH 6. 03 so that the total volume would be 20 ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

[Example 1]

Effects of TCF-II in septic rats made by puncturing cecum

[0026] Septic model animals were made by puncturing cecum once with 22G injection needle using 7 week old male SD rats (J. Surgical Research 29, 189-201 (1980)). At 48 hours before making septic model, animals were divided into two groups consisting of a control group which would be administered with vehicle only (vehicle-administered group) (n=15) and a TCF-II-administered group (n=11). They were intravenously administered with vehicle or TCF-II (1000 μ g/kg) 5 times every 12 hours. The result of the change of survival rate of animals until 2 days after making septic model was shown in figure 1. As the results, the survial rate on day 2 of TCF-II administered group was significantly higher than that of vehicle administered group, which demonstrated that TCF-II administration significantly increased in the survival rate of septic rats. That is, it was confirmed that TCF-II improved the survival rate of individuals with sepsis.

[Example 2]

Effects of TCF-II administration on LPS-induced bacterial translocation

[0027] LPS was intravenously administered in 7 weeks old male ICR mice (5mg/kg). At 30 hours before LPS administration, animals were divided into 2 groups consisting of a control group which would be administered with vehicle only (vehicle-administered group) (n=6) and TCF-II-administered group (n=6). They were intravenously administered with vehicle or TCF-II (200 μ g/kg) 3 times every 12 hours. The result of the number of bacteria detected in mesenteric lymph node at 24 hours after LPS administration was shown in figure 2. The detected number of bacteria in the TCF-II-administered group was significantly lower than that of the control group, which demonstrated that TCF-II administration significantly suppressed LPS-induced translocation of bacteria from intestine. That is, it was confirmed that TCF-II significantly improved the survival rate against bacterial translocation which can be a trigger of inducing sepsis.

Industrial utility

[0028] By the present invention, a therapeutic agent for preventing and/or treating sepsis comprising TCF-II as an effective ingredient is provided. The therapeutic agent of the present invention is useful for preventing and/or treating sepsis caused by more than one factor selected from the group consisting of infectious disease, burn, surgery, cancer, AIDS, radiotherapy, chemotherapy, long term TPN.

Claims

1. A therapeutic agent for preventing and/or treating sepsis, comprising Tumor Cytotoxic Factor-II (TCF-II) as an effective ingredient.

Figure 1

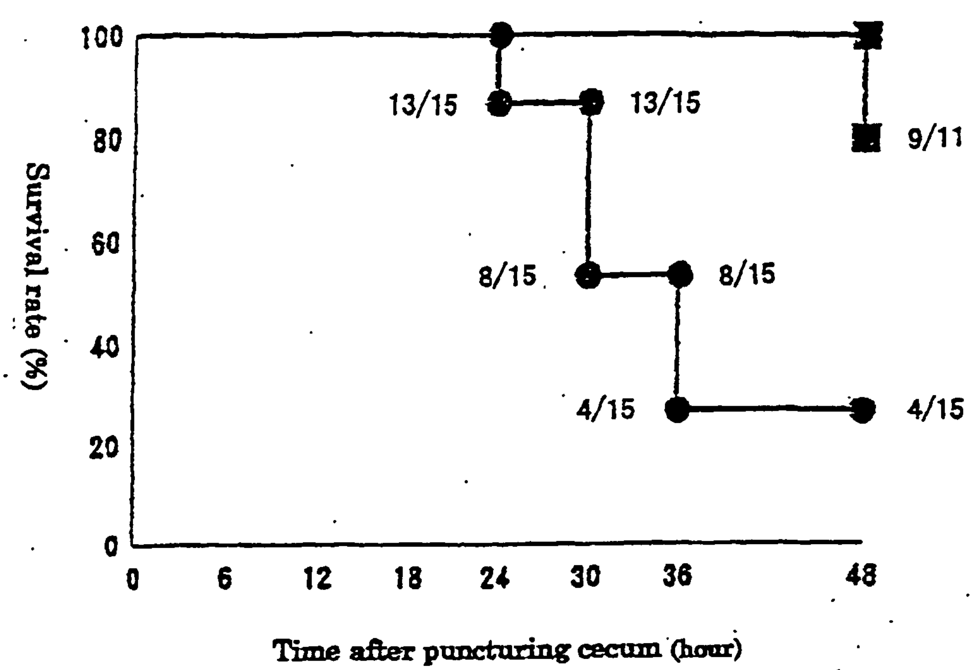
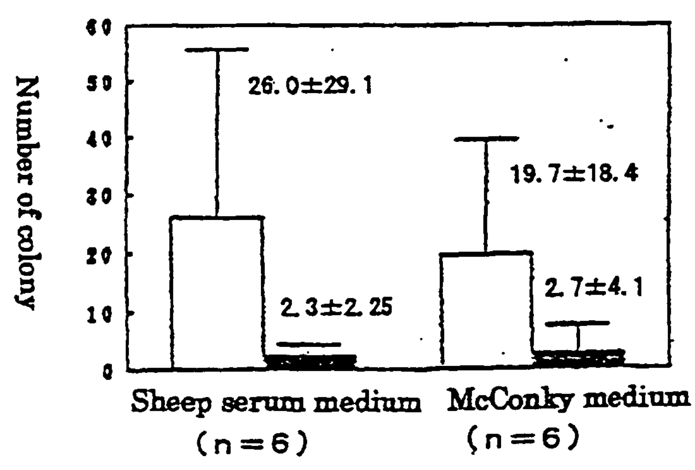


Figure 2



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP99/01373

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl⁶ A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl⁶ A61K38/17

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAPLUS (STN), MEDLINE (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, 90/10651, A1 (Snow Brand Milk Products Co., Ltd.), 20 September, 1990 (20. 09. 90) & EP, 462277, A1 & JP, 7-68272, A & US, 5587359, A	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 May, 1999 (25. 05. 99)	8 June, 1999 (08. 06. 99)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)